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Sugar-Dependent Gibberellin-Induced Chalcone Synthase Gene Expression in Petunia Corollas[1]

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The induction of anthocyanin synthesis and anthocyanin biosynthetic gene expression in detached petunia (*Petunia hybrida*) corollas by gibberellic acid (GA_3) requires sucrose. Neither sucrose nor GA_3 alone can induce these processes. We found that GA_3 enhances sucrose uptake by 20 to 30%, and we tested whether this is the mechanism by which the hormone induces gene expression. Changing the intracellular level of sucrose with the inhibitors *p*-chloromercuribenzenesulfonic acid and vanadate did not inhibit the induction of chalcone synthase gene (*chs*) expression by GA_3. Growing detached corollas in various sucrose concentrations did not affect the induction of the gene but did affect its level of expression and the level of anthocyanin accumulated. Only metabolic sugars promoted GA_3-induced anthocyanin accumulation. Mannitol and sorbitol had no effect and 3-*O*-methylglucose only slightly promoted *chs* expression and anthocyanin accumulation. Our results do not support the suggestion that sugars act as specific signals in the activation of anthocyanin biosynthetic gene expression during petunia corolla development. We suggest that sugars are essential as general sources of carbohydrates for carbon metabolism, upon which the induction of pigmentation is dependent.

Flowers of most plants are heterotrophic and require imported carbohydrates for their development. In most cases, Suc is transported to the flower from leaves or storage organs. The flower bud is a major sink for assimilates under favorable growth conditions, whereas a shortage of carbohydrates often leads to the arrest of flower development (Halevy, 1987). The role of sugars in flower development is multifunctional: they can act as energy sources, as osmotic regulators, and as precursors for metabolic processes (Kuiper et al., 1991).

Sugars play a central role in the regulation of petunia (*Petunia hybrida*) flower development, which requires both Suc and GA. Normal development and pigmentation of detached, intact petunia flowers can proceed only when Suc is supplied (Weiss et al., 1995). However, when the anthers are removed from the flowers, growth and anthocyanin accumulation are inhibited. These processes are restored upon application of GA_3 (Weiss and Halevy, 1989). When detached corollas are grown in vitro, they elongate and become pigmented only in the presence of both Suc and GA_3 (Weiss et al., 1992). The induction of corolla pigmentation results from the activation of anthocyanin biosynthetic genes, including *chs*, the chalcone-flavanone isomerase gene, the dihydroflavonol 4-reductase gene, and the anthocyanidin synthase gene (Weiss et al., 1993, 1995). The promotive effect of GA_3 and Suc is not exclusive to anthocyanin biosynthetic genes; it also includes other general metabolic genes in the corolla such as the triose-P isomerase gene (Ben-Nissan and Weiss, 1995) and the *S*-adenosylmethionine synthetase gene (Izhaki et al., 1996). We found that Glc but not mannitol can replace Suc in the induction of *chs* expression (Weiss et al., 1992). We therefore propose that sugars are required as a carbohydrate source for general carbon metabolism, the latter being essential for these processes.

Suc may also induce *chs* expression directly. Tsukaya et al. (1991) showed that sugars induce the transcription of the petunia *chs*-A gene in transgenic Arabidopsis and suggested that *chs* expression in petunia flowers is regulated by the intracellular level of sugars. Since our previous results showed that Suc alone does not induce *chs* expression and anthocyanin synthesis, but requires the presence of GA_3, we examined the possibility that GA_3 promotes Suc uptake by the detached corollas and that sugar activates gene expression directly. Induction of Suc uptake by GA_3 has been found in several flowers, including rose (Kuiper et al., 1991) and tulip (Lukaszewska, 1995), as well as in subhooks of etiolated pea (*Pisum sativum*) seedlings (Miyamoto et al., 1993). In the present work we examined the effect of GA_3 on sugar uptake by detached petunia corollas and studied the role of sugar in GA_3-induced anthocyanin synthesis and *chs* expression.

MATERIALS AND METHODS

Petunia (*Petunia hybrida*) plants were grown under normal greenhouse conditions. The VR hybrid (V23 × R51) was used throughout the experiments. Flower development was divided into seven stages (Weiss and Halevy, 1989); flower buds used for this study were at developmental stage 3 (corollas of 1.4–1.7 cm in length).

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Abbreviations: *chs*, chalcone synthase gene; PCMBS, *p*-chloromercuribenzenesulfonic acid.

In Vitro Culture of Corollas and Anthocyanin Determination

Corollas were detached and separated from the anthers, and the limbs were grown in vitro, as described previously (Weiss and Halevy, 1989). Corolla limbs were grown in tubes containing 5 mL of 150 mM solutions of each of the various sugars (as specified in "Results") in deionized water, with or without the addition of GA_3 (Sigma) and the inhibitors PCMBS (Sigma) and vanadate (Aldrich). Corolla pigmentation was quantified by measuring the amount of anthocyanins, as described previously (Weiss and Halevy, 1989).

RNA Extraction and Northern Blot Analysis

Total RNA was extracted from corolla limbs according to the method of van Tunen et al. (1988). RNA (10 μg) was size-fractionated on a formaldehyde-denaturating agarose gel (Maniatis et al., 1982) and blotted onto a Hybond-N+ filter (Amersham, UK). Following electrophoresis, the formaldehyde gel was briefly stained with ethidium bromide and photographed before blotting to ensure that equal amounts of RNA had been used for each sample. The blots were hybridized with ^{32}P-labeled, full-size petunia cDNA probes for *chs* (Koes et al., 1986). Following hybridization, the blots were washed twice with 0.3 M NaCl, 30 mM sodium citrate, pH 7.0 (2× SSC), and 0.1% (w/v) SDS at 60°C for 10 min and then autoradiographed.

Sugar Uptake by Excised Corollas

Detached corolla limbs were incubated in a 150 mM Suc solution, as described previously (Weiss and Halevy, 1989). After 24 h, [^{14}C]Suc, [^{14}C]Glc, or [^{14}C]mannitol (Amersham, UK) at a specific activity of 33 MBq mol^{-1} was added to a final activity of 7.4 kBq mL^{-1}. Hormones or inhibitors were added 2 h later. Samples were taken periodically and washed three times for 5 min each with the appropriate sugar solution at 150 mM. Corollas were extracted in 0.5 mL of tissue solubilizer (Soluene 350, Hewlett-Packard) for 72 h, after which radioactivity was measured as described previously (Weiss and Halevy, 1989).

RESULTS

Suc Is Required for GA_3-Induced Anthocyanin Synthesis

We previously showed that Suc is required for the activation of *chs* expression by GA_3 (Weiss et al., 1992). To study whether Suc is also required for the induction of anthocyanin accumulation by the hormone, we detached corollas at stage 3 and incubated them in water, 10 μM GA_3, 150 mM Suc, or Suc with GA_3. After 48 h, tissues were extracted for anthocyanin determination.

Incubation of detached corollas in water, Suc alone, or GA_3 alone had no effect on anthocyanin content (Fig. 1). However, when GA_3 and Suc were supplied together, a high level of anthocyanin, about 4 times that found with the other treatments, was observed. These results show that Suc is required for GA_3-induced anthocyanin synthesis.

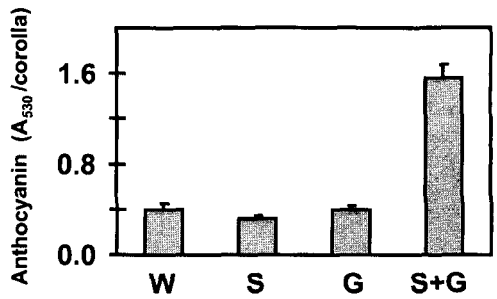

Figure 1. Anthocyanin accumulation in detached corollas. Corollas were detached and incubated in water (W), 150 mM Suc (S), 10 μM GA_3 (G), or Suc and GA_3 (S+G). After 48 h, anthocyanin was extracted and measured. Values are means ± SE of seven replications.

Sugar Uptake by Detached Corollas

GA_3 may promote Suc uptake by corolla cells, leading to the activation of anthocyanin biosynthetic gene transcription. To test this possibility, we first incubated detached corollas in 150 mM Suc for 24 h to eliminate the effect of endogenous GAs (Weiss et al., 1992, 1995) and then added [^{14}C]Suc. GA_3 was added to the medium 2 h later and samples of GA_3-treated and nontreated corollas were taken periodically for radioactivity measurements. The level of radioactivity increased with time with or without GA_3 in the growing medium (Fig. 2A) and was almost linear for up to 24 h (data not shown). GA_3 enhanced Suc uptake, an effect observed as early as 15 min after its application (Fig. 2A). The level of radioactivity found in GA_3-treated corollas was always 20 to 30% higher than that in nontreated corollas.

We then tested the effect of various GA_3 concentrations on Suc uptake. Detached corollas were treated as described except that various concentrations of GA_3 were used, ranging from of 0 to 100 μM. After 2 h of incubation with the hormone, tissues were taken for radioactivity measurements. The promotive effect of GA_3 on Suc uptake was dependent on its concentration (Fig. 2C); a maximal effect was observed at 10 μM, whereas at the higher concentration of 100 μM, a lower promotive effect was observed.

Suc uptake is an active process, and as such should be temperature-dependent (Williams et al., 1990). To test the effect of temperature on Suc uptake, we conducted the above experiment at both 4 and 25°C. Incubating corollas at the low temperature inhibited Suc uptake in both the presence and absence of GA_3 (Fig. 2B). The level of Suc taken up by corollas that were treated with both Suc and GA_3 at 25°C was about 2-fold that at 4°C. These results support our assumption that Suc is taken up by cells via an active process.

We also examined the effect of GA_3 on the uptake of other sugars. We incubated detached corollas in 150 mM Suc, Glc, or mannitol for 24 h and then added [^{14}C]Suc, [^{14}C]Glc, or [^{14}C]mannitol. GA_3 was added to the growth medium 2 h later, and after an additional 2 h, GA_3-treated

and nontreated corollas were sampled for radioactivity measurements. GA_3 enhanced Suc and Glc uptake, with the radioactivity levels found in both treatments in the presence of GA_3 being about 20% higher than those found in the sugar controls (Fig. 2D). The level of radioactivity found in corollas treated with mannitol was low and was not affected by GA_3. This suggests that Suc and Glc, but not mannitol, are taken up by corolla cells.

Suc Uptake Is Not Directly Related to *chs* Induction

We examined the effect of Suc concentrations in the growth medium on Suc uptake. Detached corollas were incubated in the presence of various Suc concentrations, from 0 to 150 mM. After 24 h, [^{14}C]Suc was added, and 2 h later, GA_3 was added in half of the samples to a final concentration of 10 μM. Corollas were incubated for an additional 2 h and then radioactivity was measured. Increasing Suc concentration in the solution promoted Suc uptake, and GA_3 further enhanced it by 20 to 30% (Fig. 3A). The amount of Suc taken up by corollas held at low Suc concentrations with or without GA_3 was much lower than that taken up by corollas that were held at high Suc concentrations with or without GA_3.

If GA_3 induces *chs* expression by increasing the intracellular level of Suc, then at a low Suc concentration, GA_3 would not be expected to induce the process. To test this assumption, we incubated detached corollas in several Suc concentrations with or without GA_3 and determined anthocyanin levels 48 h later. The content of anthocyanin in corollas held without GA_3 was low and was not affected by the concentration of Suc in the medium (Fig. 3B). When corollas were grown in the presence of GA_3, a dose-dependent response to Suc concentration was evident. As Suc concentration increased, the promotive effect of GA_3 on anthocyanin accumulation was enhanced (Fig. 3B), reaching a maximum at 300 mM (data not shown). We next examined the effect of Suc concentration on *chs* expression. Corollas were first incubated in the presence of Suc concentrations ranging from 15 to 150 mM for 24 h and then GA_3 was added. After a further incubation for 24 h, the corollas were extracted and *chs* mRNA was analyzed. The expression of *chs* was induced by GA_3 at all of the Suc concentrations tested and the highest transcript level was found in corollas treated with 75 mM Suc (Fig. 3C). These results suggest that the level of Suc in the cells does not affect *chs* induction but is related to the intensity of GA_3 action, both on *chs* expression and anthocyanin synthesis.

Further evidence supporting our contention that GA_3 does not induce *chs* expression directly via the promotion of Suc uptake by corolla cells was obtained from experiments in which we tested the effects of PCMBS, a sulfydryl-modifying reagent (Williams et al., 1990), and vanadate, an inhibitor of plasma membrane H^+-ATPase (DuPont et al., 1981). Detached corollas were incubated in 150 mM Suc and after 22 h vanadate or PCMBS was added to a final con-

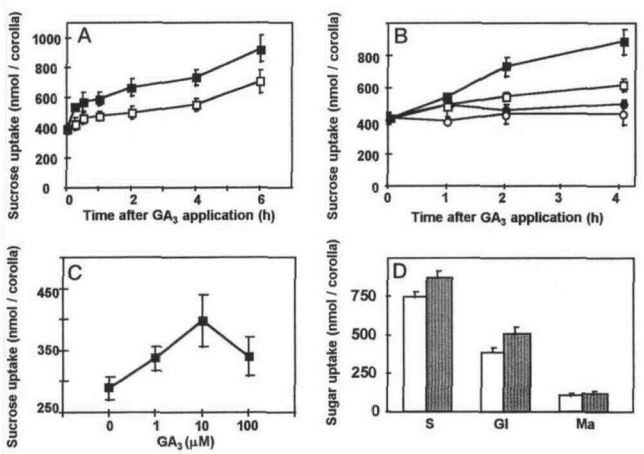

Figure 2. Sugar uptake by detached corollas. A, Detached corollas were incubated in 150 mM Suc for 24 h and then [^{14}C]Suc was added with (■) or without (□) 10 μM GA_3. After the specified times, samples were taken for radioactivity measurements. Values are means ± SE of 15 replications. B, Detached corollas were incubated in 150 mM Suc for 24 h and then [^{14}C]Suc was added. Tissues were then incubated at 25°C with (■) or without (□) 10 μM GA_3 or at 4°C with (●) or without (○) GA_3. At the specified times, samples were taken for radioactivity measurements. Values are means ± SE of 10 replications. C, Detached corollas were incubated in 150 mM Suc for 24 h and then [^{14}C]Suc was added with the specified concentration of GA_3. After 2 h tissues were taken for radioactivity measurements. Values are means ± SE of 10 replications. D, Detached corollas were incubated in 150 mM Suc (S), Glc (Gl), or mannitol (Ma) for 24 h and then [^{14}C]Suc, [^{14}C]Glc, or [^{14}C]mannitol was added with (stippled column) or without (empty column) 10 μM GA_3. After 2 h, samples were taken for radioactivity measurements. Values are means ± SE of 15 replications.

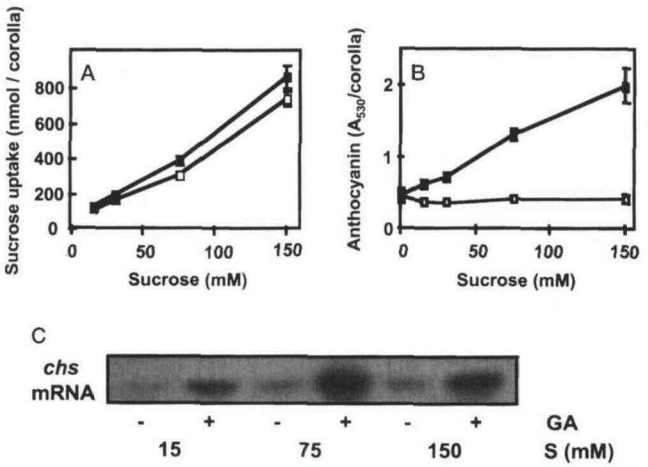

Figure 3. Effect of Suc concentration on Suc uptake, anthocyanin accumulation, and *chs* expression. A, Detached corollas were incubated in different Suc concentrations for 24 h and then [^{14}C]Suc was added with (■) or without (□) 10 μM GA_3. After 2 h samples were taken for radioactivity measurements. Values are means ± SE of 15 replications. B, Detached corollas were incubated in different Suc concentrations with (■) or without (□) 10 μM GA_3. After 48 h tissues were taken for anthocyanin determination. Values are means ± SE of 7 replications. C, Detached corollas were incubated in different Suc (S) concentrations for 24 h and then 10 μM GA_3 (GA) was added. After 24 h RNA was extracted from GA_3-treated (+) and nontreated (−) corollas. Equal amounts of RNA, as evident by ethidium bromide staining, were loaded and *chs* mRNA levels were analyzed.

centration of 0.5 and 1 mM, respectively. [^{14}C]Suc was added 2 h later, and after a further 2 h, GA_3 was added. The corollas were incubated for an additional 2 h and then radioactivity was measured. Both PCMBS and vanadate inhibited Suc uptake by about 40% and completely abolished the promotive effect of GA_3 on this process (Fig. 4A).

To examine whether these inhibitors also affect *chs* induction by GA_3, we incubated detached corollas in a 150 mM Suc solution for 24 h, then we added PCMBS or vanadate, and 2 h later we added GA_3. RNA was extracted after a short period (6 h) of incubation, and *chs* expression was analyzed. Neither inhibitor eliminated the induction of *chs* expression by GA_3 (Fig. 4, C and D). PCMBS slightly reduced the level of *chs* expression, whereas vanadate had no effect. These results show that, although PCMBS and vanadate completely inhibited GA_3-promoted Suc uptake, they did not do the same to gene induction. In several experiments, PCMBS and vanadate were added with Suc but without GA_3 and had only a small, insignificant effect on the basal levels of *chs* expression (Fig. 4, C and D). However, these effects were not reproducible in other experiments (data not shown).

We also tested the effect of the inhibitors on GA_3-induced anthocyanin accumulation. Detached corolla limbs were incubated in the same solutions as before (Fig. 4A), and after 48 h, anthocyanin was measured. Vanadate partially inhibited and PCMBS completely abolished the effect of the hormone on pigment accumulation (Fig. 4B). However, it should be emphasized that anthocyanin accumulation was measured after rather long periods of incubation (48 h). Since PCMBS affects much of the existing sulfhydryl groups in the cell and vanadate inhibits the overall proton-pumping activity of the plasmalemma, both chemicals are probably phytotoxic after such long incubation periods and, therefore, their inhibitory effects on pigment accumulation are not specific for GA_3 action.

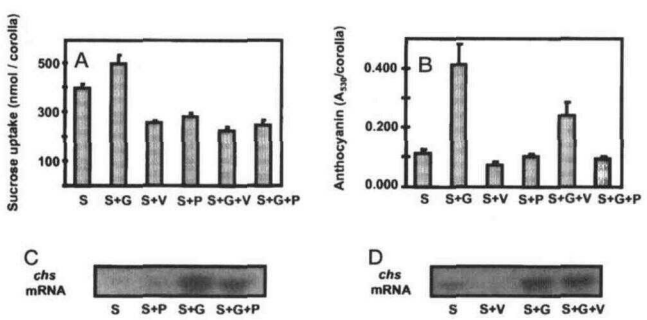

Figure 4. Effect of PCMBS and vanadate on Suc uptake, anthocyanin accumulation, and *chs* expression. A, Detached corollas were incubated in 150 mM Suc, and after 22 h, 1 mM PCMBS (P) or 0.5 mM vanadate (V) was added. [^{14}C]Suc was added 2 h later with (G) or without 10 μM GA_3. Corollas were incubated for a further 2 h and then taken for radioactivity measurements. Values are means ± SE of 10 replications. B, Detached corollas were incubated in the same solutions as before (as in A) and after 48 h the tissues were taken for anthocyanin determination. Values are means ± SE of 7 replications. C and D, Detached corollas were incubated in Suc (150 mM) for 24 h. C, +1 mM PCMBS (P); D, +0.5 mM vanadate (V). After 2 h, 10 μM GA_3 (G) was added, and 6 h later RNA was extracted from GA_3-treated (+) and nontreated (−) corollas. Equal amounts of RNA, as evident by ethidium bromide staining, were loaded and *chs* mRNA levels were analyzed.

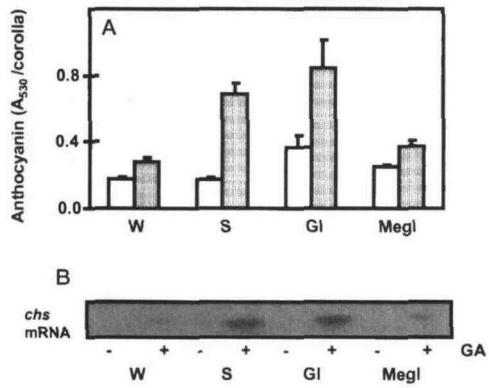

Figure 5. Effect of 3-*O*-methylglucose on anthocyanin accumulation and *chs* expression. A, Detached corollas were incubated in water (W), 150 mM Suc (S), 150 mM Glc (Gl), or 150 mM 3-*O*-methylglucose (Megl) with (stippled column) or without (empty column) 10 μM GA_3. After 48 h tissues were taken for anthocyanin determination. Values are means ± SE of 10 replications. B, Corollas were detached and incubated for 24 h in water (W) or in 150 mM Suc (S), Glc (Gl), or 3-*O*-methylglucose (Megl) and then transferred to the same solutions with (+) or without (−) 10 μM GA_3. After 24 h RNA was extracted from the corollas. Equal amounts of RNA, as evident by ethidium bromide staining, were loaded and *chs* mRNA levels were analyzed.

Metabolic Sugars Are Required for Both *chs* Expression and Anthocyanin Synthesis

We showed previously that Glc but not mannitol acts similarly to Suc in the induction of *chs* expression (Weiss et al., 1992). Mannitol was not taken up by the corollas (Fig. 2D) and apparently does not play a role in regulating the osmotic potential of corolla cells. We incubated detached corollas with 3-*O*-methylglucose, a nonmetabolic derivative of Glc that has been previously shown to be taken up by plant cells and to modify their osmotic potential (Colombo et al., 1978). Corollas were incubated for 48 h with or without GA_3 in water, Suc, Glc, or 3-*O*-methylglucose and then anthocyanin levels were determined. As before, GA_3 promoted anthocyanin accumulation when applied with Suc or Glc (Fig. 5A). In contrast, when the corollas were incubated with 3-*O*-methylglucose, only a small effect was observed. Nevertheless, this effect was slightly higher than that of water alone.

We also tested the effect of 3-*O*-methylglucose on *chs* induction by GA_3. Detached corollas were incubated for 24 h in water or in a Suc, Glc, or 3-*O*-methylglucose solution and then transferred to similar solutions with or without GA_3. After a further 24 h, the tissues were analyzed for *chs* expression. GA_3 induced *chs* expression when applied with Suc or Glc (Fig. 5B); however, when it was applied with water alone or with 3-*O*-methylglucose, only a low level of expression was found. These results suggest that modifications of cell osmotic potential play only a minor

role, if any, in the effect of sugars on GA_3-induced *chs* expression.

We tested the effect of other sugars on GA_3-induced anthocyanin accumulation. Detached corollas were incubated in water or in 150 mM solutions of various sugars with or without GA_3, and after 48 h, anthocyanin contents were measured. Only metabolic sugars enhanced GA_3-induced anthocyanin accumulation; the nonmetabolic mannitol and sorbitol had no effect (Fig. 6). All of the metabolic sugars promoted GA_3 induction to various degrees. The most effective sugar was Glc. Suc and Gal had lower, intermediate effects, and Fru, trehalose, and inositol had the lowest, albeit significant effects.

DISCUSSION

The induction of anthocyanin synthesis and anthocyanin biosynthetic gene expression by GA_3 in detached petunia corollas requires the presence of sugar in the growth medium. Sugars may serve as specific signals for the activation of specific genes, as cellular osmotic regulators, or as a general energy source for carbon metabolism in the developing flower.

In this work we first examined the possibility that GA_3 enhances the intracellular level of Suc, which in turn activates the expression of *chs* and other genes. In a previous study we monitored the rate of Suc uptake by fresh corollas exposed to [^{14}C]Suc immediately after detachment (Weiss and Halevy, 1989). In that study we did not find enhancement of Suc uptake following GA_3 application. In later studies we found that corollas at stage 3 contain high levels of endogenous GAs (Weiss et al., 1995) and that the response to exogenous GA_3 could be measured only after several hours of incubation (Weiss et al., 1992). Accordingly, in the present study we used an alternative system in which detached corolla limbs were first incubated for 24 h in a Suc solution to eliminate the possible effects of endogenous GAs. Only then did we add GA_3 to study its effects. Using this experimental system, we found that GA_3 promoted Suc uptake by 20 to 30% (Fig. 2A) in a concentration-dependent manner,

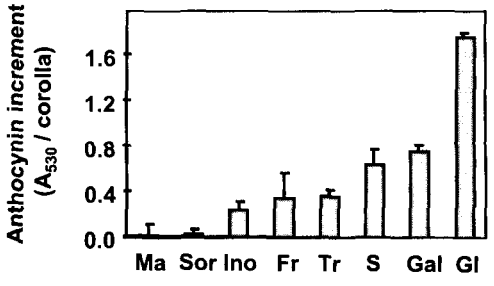

Figure 6. Effect of different sugars on GA_3-induced anthocyanin accumulation. Detached corollas were incubated in water or 150 mM mannitol (Ma), sorbitol (Sor), inositol (Ino), Fru (Fr), trehalose (Tr), Suc (S), Gal (Gal), or Glc (Gl), with or without 10 μM GA_3. After 48 h corollas were taken for anthocyanin measurements. The anthocyanin increment was calculated as the difference in A_{530} between GA_3-treated and nontreated corollas. Values are means ± SE of 10 replications.

with a maximal effect at 10 μM; lower and higher hormone concentrations had smaller effects. Similar responses to GA_3 concentration were also found for *chs* expression and anthocyanin accumulation (D. Weiss, unpublished results). Thus, a positive correlation was revealed between the effects of GA_3 on Suc uptake and on *chs* expression and pigmentation, raising the possibility of their interrelationship. However, several pieces of evidence indicated that the increased intracellular level of Suc, which was promoted by GA_3, is not the mechanism by which the hormone activates *chs* in the corolla. The level of Suc taken up by corollas grown in high Suc concentrations without GA_3 was much higher than the level taken up by corollas grown in low Suc concentrations with GA_3 (Fig. 3A). However, whereas *chs* expression was not induced at high sugar concentrations in the absence of GA_3, it was induced at low Suc concentrations in the presence of the hormone. Furthermore, the inhibitors PCMBS and vanadate, which completely abolished GA_3-promoted Suc uptake (Fig. 4A), did not promote the induction of *chs* by the hormone. These results suggest that GA_3 promotes *chs* expression and Suc uptake independently.

Sugars can regulate gene transcription by signaling a specific receptor that leads to the synthesis of *trans*-acting regulatory proteins (Kim et al., 1991). The possibility that sugars play a similar role in the activation of *chs* expression is supported by studies of Arabidopsis and soybean leaves showing that sugars regulate *chs* expression directly (Tsukaya et al., 1991; Sadka et al., 1994). In addition, Tsukaya et al. (1991) reported the existence of a region within the petunia *chs* promoter that shares homology with the "Suc box" of the sweet potato sporamin promoter and suggested that the petunia *chs* gene is regulated in flowers by intracellular sugar levels. Our results, however, do not support this suggestion. An increased intracellular level of Suc was not sufficient to induce *chs* expression; it required the presence of GA_3. Kim et al. (1991) showed that the tobacco PI-II gene is induced in leaves by wounding and that this effect is enhanced by sugar, whereas sugar alone had no effect on intact leaves. Accordingly, they suggested the existence of two *cis* elements within the PI-II promoter: a wound- and a sugar-responsive element. They also suggested that the sugar-responsive element is an enhancer, not an inducer.

Sugar may play a similar role in petunia corollas: it does not induce *chs* expression on its own, but it enhances the effect of GA_3, perhaps by inducing a specific *trans*-acting factor, which, in turn, binds to the sugar box in the *chs* promoter. Our results indeed show that changes in the intracellular level of Suc affect GA_3-induced *chs* expression and anthocyanin accumulation. When detached corollas were grown in various Suc concentrations complemented with GA_3, a maximal level of *chs* expression was found at 75 mM Suc (Fig. 3C), and a maximal accumulation of anthocyanin was found at 300 mM (data not shown). The saturation of these responses at such high concentrations does not support the possibility that sugars act as a signal. Moreover, several sugars were found to affect pigmentation similarly (Fig. 6): the possibility that any of them could serve as a specific signal is small. Nevertheless, one of the

sugar degradation products may directly induce or enhance *chs* expression.

Sugars promote flower development by regulating the osmotic potential of the cells (Halevy and Mayak, 1981). The results of the present study suggest that sugars may have a limited role in the promotion of GA_3-induced gene expression and pigmentation via modifications of the osmotic potential of corolla cells. In addition to the effects of metabolic sugars that are taken up by the cells and were found to induce anthocyanin synthesis (Fig. 6), the results of an experiment using the nonmetabolized Glc derivative 3-*O*-methylglucose at least partially support this suggestion. This compound is known to be taken up by cells (Colombo et al., 1978) and to modify their osmotic potential. When applied with GA_3, it slightly promoted *chs* expression and anthocyanin accumulation. This suggests that modifications in the osmotic potential of the cells, mediated by sugars, may create a condition that facilitates the GA activation of *chs* expression in developing corollas. Still, it is possible that the methylglucose is partially demethylated and, consequentially, the free Glc enters the carbohydrate metabolism. This possibility, however, is not supported by any experimental results.

Our results provide indirect support for the suggestion that the main role of sugar in the enhancement of GA_3 responses is as a source of carbohydrates for carbon metabolism, probably to be used for energy. It should be noted that in petunia corollas GA_3 induces not only the expression of the anthocyanin biosynthetic genes but also that of several other genes. For example, we recently reported that GA_3 induces expression of the glycolytic triose-P isomerase gene (Ben-Nissan and Weiss, 1995) and the *S*-adenosylmethionine synthetase gene (Izhaki et al., 1996) in petunia corollas. The induction of various genes from different metabolic pathways probably requires the activation and synthesis of many different transcriptional factors (Weiss et al., 1992). Indeed, several *myb*-type regulatory genes have been identified in petunia corollas and their expression is induced by GA_3 (Mur, 1995). The induction of many regulatory and structural genes and of protein synthesis may depend on the availability of large sources of energy. We propose that this is the main function of sugars in this process.

In conclusion, in this study we showed that sugars are required for GA_3-induced *chs* expression and anthocyanin synthesis in petunia corollas. Although GA_3 promotes Suc uptake, this is probably not the mechanism by which the hormone affects *chs* expression. Our results support the possibility that sugars are essential as a general source of carbohydrates for this process.

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LITERATURE CITED

Ben-Nissan G, Weiss D (1995) Developmental and hormonal regulation of a triosephosphate isomerase gene in petunia corollas. J Plant Physiol **147:** 58–62

Colombo R, De-Michelis MI, Lado P (1978) 3-*O*-Methyl glucose uptake stimulation by auxin and by fusicoccin in plant materials and its relationship with proton extrusion. Planta **138:** 249–256

DuPont FM, Burke LL, Spanswick RM (1981) Characterization of a partially purified adenosine triphosphatase from a corn root plasma membrane fraction. Plant Physiol **67:** 59–63

Halevy AH (1987) Assimilate allocation and flower development. *In* JG Atherton, ed, Manipulation of Flowering. Butterworth, London, pp 363–378

Halevy AH, Mayak S (1981) Senescence and post harvest physiology of cut flowers—part II. Hortic Rev **3:** 59–143

Izhaki A, Shoseyov O, Weiss D (1996) Temporal, spatial and hormonal regulation of the S-adenosylmethionine synthetase gene in petunia. Physiol Plant **97:** 90–94

Kim SR, Costa MA, An G (1991) Sugar response element enhances wound response of potato proteinase inhibitor II promoter in transgenic tobacco. Plant Mol Biol **17:** 973–983

Koes RE, Spelt CE, Reif HJ, van der Elsen PJM, Veltkamp E, Mol JNM (1986) Floral tissue of *Petunia hybrida* (V30) expresses only one member of the chalcone synthase multigene family. Nucleic Acids Res **14:** 5229–5239

Kuiper D, van Reenen HS, Ribot SA (1991) Effect of gibberellic acid on sugar transport into petals of "Madelon" rose flowers during bud opening. Acta Hortic **298:** 93–95

Lukaszewska AJ (1995) Distribution of sugars in tulip flower parts as affected by ethrel and GA_3 in the holding solution. Acta Hortic **405:** 351–355

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Miyamoto K, Ueda J, Kamisaka S (1993) Gibberellin-enhanced sugar accumulation in growing subhooks of etiolated *Pisum sativum* seedlings. Effect of gibberellic acid, indolacetic acid and cycloheximide on invertase activity, sugar accumulation and growth. Physiol Plant **88:** 301–306

Mur L (1995) Characterization of members of the *myb* gene family of transcription factors from *Petunia hybrida.* PhD thesis. Vrije Universitait, Amsterdam, The Netherlands

Sadka A, DeWald DB, May GD, Park WD, Mullet JE (1994) Phosphate modulates transcription of soybean *vspB* and other sugar-inducible genes. Plant Cell **6:** 737–749

Tsukaya H, Ohshima T, Naito S, Chino M, Komeda Y (1991) Sugar-dependent expression of the *CHS-A* gene for chalcone synthase from petunia in transgenic *Arabidopsis.* Plant Physiol **97:** 1414–1421

van Tunen AJ, Koes RE, Spelt CE, van der Krol AR, Stuitje AR, Mol JNM (1988) Cloning of the two chalcone flavanone isomerase genes from *Petunia hybrida:* coordinate, light-regulated and differential expression of flavonoid genes. EMBO J **7:** 1257–1263

Weiss D, Halevy AH (1989) Stamens and gibberellin in the regulation of corolla pigmentation and growth in *Petunia hybrida.* Planta **179:** 89–96

Weiss D, van Blokland R, Kooter JM, Mol JNM, van Tunen AJ (1992) Gibberellic acid regulates chalcone synthase gene transcription in the corolla of *Petunia hybrida.* Plant Physiol **98:** 191–197

Weiss D, van der Luit A, Knegt E, Vermeer E, Mol JNM, Kooter JM (1995) Identification of endogenous gibberellins in petunia flower, induction of anthocyanin biosynthetic gene expression, and the antagonistic effect of abscisic acid. Plant Physiol **107:** 695–702

Weiss D, van der Luit AH, Kroon JTM, Mol JNM, Kooter JM (1993) The petunia homologue of the *Antirrhinum majus candi* and *Zea mays A2* flavonoid genes; homology to flavonone 3-hydroxylase and ethylene-forming enzyme. Plant Mol Biol **22:** 893–897

Williams LE, Nelson SJ, Hall JL (1990) Characterization of solute transport in plasma membrane vesicles isolated from cotyledons of *Ricinum communis* L. Planta **182:** 540–545